

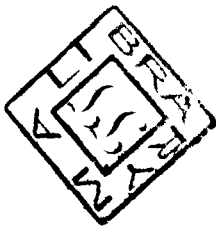


STUDIES ON THE INTERACTION OF PATHOGENIC ORGANISMS

**DISSERTATION SUBMITTED
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
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IN
BOTANY**

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I N T R O D U C T I O N
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R E V I E W O F L I T E R A T U R E

Plant parasitic nematodes, though microscopic, are responsible for causing considerable economic losses. There is paucity of information as to the exact amount of losses which plant parasitic nematodes cause, however whatever information there is available, indicates that they are bane in crop production. It has been estimated that they are responsible of causing as much as 10% crop loss annually (Annon, 1971). In England and Wales in 1949, potato cyst nematode, Heterodera rostochiensis Wollenweber, 1923 resulted in a loss of about 200,000 tones of potatoes costing about 20 million pounds (Soutney and Samuel, 1954). In the state of California alone annual losses due to nematode attack were estimated of the order of 90 million dollars (Allen and Maggenti, 1959). In the U.S.A., according to Cairns (1955); Hutchinson et al. (1961) and Taylor (1967), crops worth \$ 500,000,000; \$ 250,000,000 and \$ 372,335,000 were lost annually due to plant parasitic nematodes. In a recent report Feldmeier et al. (1971) estimated annual losses due to nematodes to the tune of \$ 1,038,374,300 for field crops; \$ 225,145,900 for fruits and nut crops; \$ 266,989,100 for vegetable crops and \$ 59,817,634 for ornamental crops.

In India Van Berkum and Seshadri (1970) reported annual losses due to "Ear-cockle" disease caused by Anaula tritici (Steinbuch, 1799) Chitwood, 1935 on wheat amounting

to 10 million dollars; due to "Molya" disease caused by Heterodera avenae Wollenweber, 1924 on barley to 8 million dollars and due to Pratylenchus coffeae (Zimmermann, 1898) Goodey, 1951 on coffeae to 3 million dollars.

In nature plants are exposed to numerous micro-organisms which form the common components of soil biosphere forming a variety of relationship including synergistic. In such multipathogenic conditions the losses to plants are much more than in monopathogenic conditions. Although the exact figures for such situations are lacking but observations by various workers show that they are responsible to heavy losses. Miller (1968) reported that black-shank resistant variety of tobacco suffered 64% with black-shank fungus, Phytophthora parasitica f. nicotianae (Breda de Hann) Tucker in the presence of Meloidogyne javanica (Treub, 1885) Chitwood, 1949 as against 22% when present alone. The various aspects of multipathogenic effects on plants have been reviewed by Pitter (1963, 1965); Powell (1963, 1971 a & b); Maski and Hewitt (1963); Miller (1965) and Bergeson (1972).

Plant parasitic nematodes are often involved in disease complexes with fungi responsible for wilt, root-rots and various seedling diseases.

I. NEMATODE-FUNGUS WILT DISEASE COMPLEXES

In most of the nematode-wilt causing fungal disease interactions, Fusarium or Verticillium are involved. In 1892 Atkinson for the first time reported that root-knot and Fusarium caused distinct diseases of cotton, however, far more damage was caused when both were present in the soil. Further cultivar resistant to Fusarium became susceptible if root-knot was present in the field. Since then many reports have been published involving Fusarium or Verticillium and root-knot nematode on a variety of crops.

Martin et al. (1956) reported that out of five nematodes used (Meloidogyne incognita, M. incognita acrita, Trichodorus, Tylenchorhynchus and Helicotylenchus) only M. incognita (Kofoid and White, 1919) Chitwood, 1949 and M. incognita acrita Chitwood, 1949 could increase the severity of Fusarium wilt in cotton. A synergistic interaction between F. oxysporum (Woll.) Snyder and Hansen and M. incognita on cotton has also been reported by Perry (1961, 1963). Minton and Minton (1963), working with root-knot and Fusarium complex on cotton reported that the fungus colonized in tissues damaged by root-knot, M. incognita acrita. Holdman and Graham (1954) found that the presence of even sting nematode, Belonolaimus gracillius Steiner, 1949, resulted in breaking the resistance of cotton cultivar

resistant to Fusarium wilt. Cooper and Brodie (1962, 1963) suggested that Belonolaimus longicaudatus Rau, 1958 was as important as root-knot nematode in promoting Fusarium wilt in cotton. Neal (1954) observed greater wilt development in susceptible cotton variety than in wilt resistant variety in the presence of reniform nematode, Rotylenchulus reniformis Linford and Oliveira, 1940. These findings were confirmed by Khadr et al. (1972). There are several instances where the nematode infection has resulted in breaking of resistance. Minton and Minton (1966) found that sting nematode or root-knot nematode alone failed to adversely affect the emergence of cotton seedlings, however, severe damage was resulted in the young seedlings inoculated conjointly with the fungus and either of the two nematodes. According to Michell and Powell (1972) simultaneous inoculation with Rotylenchus brachyurus (Godfrey, 1929) Filipjev and Stekhoven, 1941 and F. oxysporum f. vasinfectum (Atk.) Snyder and Hansen caused higher percentage of wilting in Fusarium susceptible cotton plants as compared to those in which nematodes preceded the fungus by two weeks or when the fungus alone was used. Yang et al. (1976), while studying the interaction of M. incognita, Hoplolaimus galeatus (Cobb, 1913) Thorne, 1935, B. longicaudatus with F. oxysporum f. vasinfectum on wilt susceptible variety 'Nowden' cotton, reported that H. galeatus had no effect on the development of wilt.

Melendez and Powell (1965, 1967) pointed out that galled tissues of both resistant and susceptible varieties of flue-cured tobacco were more favourable sites for colonization of F. oxysporum f. nicotianae Johnson. Porter and Powell (1967) found that simultaneous inoculation of plants with F. oxysporum f. nicotianae with M. incognita or M. arenaria (Neel, 1889) Chitwood, 1949 or M. javanica, caused less damage to tobacco seedlings as compared to those in which nematode inoculation preceded the fungal inoculation by 2-4 weeks. Powell and Batten (1969) reported that association of M. incognita and Fusarium on tobacco allowed Alternaria tenuis Nees to develop on diseased plants.

Young (1939); Harrison and Young (1941) reported that the root-knot nematode greatly decreased the resistance of many tomato varieties to Fusarium wilt. Jenkins and Coursen (1957) could induce 100% wilting in Fusarium wilt resistant tomato variety "Chesapeake" in the presence of M. incognita acrita while only 60% in presence of M. hapla Chitwood, 1949. Binder and Hutchinson (1959), on the other hand, were not able to get positive results. Bowman and Bloom (1966) demonstrated indirect relationship of M. incognita to the breaking of resistance to Fusarium wilt in tomato and they were of the opinion that M. incognita appeared to change the physiology of the entire plant making it more susceptible to Fusarium wilt. Goode and

Mc Guire (1967) observed that the nematode infection pre-disposed the tomato varieties to certain wilt a-virulent races of F. oxysporum f. lycopersici (Sacc.) Snyder and Hansen and probably the fungus tended to mutate within the host. Kawamura and Hirano (1967, 1968) reported that simultaneous inoculation with M. incognita and F. oxysporum f. lycopersici caused most severe damage to the tomato seedlings. Webster (1975) also reported that Fusarium wilt of tomato was more severe in the presence of M. incognita. Miller (1975), on the other hand, found that the tobacco cyst nematode, Heterodera tabacum Lownsbery and Lownsbery, 1954 reduced the Fusarium wilt in tomato.

Thomason (1958) reported that wilt symptoms caused by F. oxysporum f. tracheiphilum (E.F. Sm.) Snyder and Hansen increased both on susceptible variety "Chino 3" and resistant variety "Grant" in the presence of M. javanica. Thomason et al. (1959) in a late publication observed that the presence of M. javanica was not only able to break the resistance of cowpea "Grant" variety to F. oxysporum f. tracheiphilum but increased the wilt to such an extent that wilt was more than on Fusarium susceptible variety "Chino 3". The preplanting fumigation with 1-2-dibromo-3 chloropropane, however, reduced wilt.

Ross (1965) observed that Fusarium oxysporum caused more damage to Soybean in the presence of Heterodera glycines

Ichinohe, 1952 as compared to M. incognita.

McGuire et al. (1958) observed that alfalfa plants showed greater percentage of wilt symptoms caused by F. oxysporum f. vasinfectum in combination with either M. hapla or M. javanica or M. incognita or M. arenaria than those by fungus alone.

Labruyere et al. (1959) reported that "Early Yellowing" disease and root-rot in pea was dependent upon the presence of both Hoplolaimus uniformis Thorne, 1949 and F. oxysporum f. pisi (Lindf.) Snyder and Hansen. Davis and Jenkins (1963) observed that the presence of M. incognita acrita and M. hapla broke the resistance of pea variety "Alaska" to F. oxysporum f. pisi race 1. An increase in wilting caused by F. oxysporum f. pisi race 2 in presence of Pratylenchus penetrans (Cobb, 1917) Chitwood and Oteifa, 1952 has also been reported by Seinhorst and Auniyasu (1971).

Newhall (1958) noticed the doubling of Fusarium wilt in banana caused by F. oxysporum f. cubens (E.F.S.) Snyder and Hansen in the presence of Radopholus similis (Cobb, 1893) Thorne, 1949 in the soil. Van Gundy and Peter (1963) observed greater reduction in growth of citrus seedlings when inoculated with citrus nematode, Tylenchulus semipene-trans Cobb, 1913 and Fusarium solani (Mart) Appel and Wr. emend. Synd. and Hans. than with either of the two alone.

Passeuliotis and Rau (1969) found that even high levels of inoculum densities of M. incognita acrita and F. oxysporum f. conglutinans (Wr.) Bynder and Hansen did not produce yellow symptoms in Fusarium resistant varieties of cabbage while in Fusarium susceptible varieties yellow symptoms appeared even at low inoculum levels.

Johnson and Littrell (1969) noticed that the presence of M. incognita, M. javanica and M. hapla failed to break the resistance of chrysanthemum variety "Ice berg" resistant to Fusarium wilt, however, their presence enhanced the wilting in susceptible variety "yellow Delaware".

Schindler et al. (1959, 1961) observed that the presence of M. hapla, M. arenaria, M. arenaria thamesi Chitwood, 1952, M. incognita, M. incognita acrita and M. javanica enhanced the severity of carnation wilt caused by F. oxysporum Schlecht f. dianthi (Prill. & Del.) Synd. & Hans. The ectoparasitic nematodes, Helicotylenchus nannus Steiner, 1945 and Rotylenchus buxophilus (Golden, 1956) Perry, 1959, on the other hand, failed to do so.

Gill (1958) stated that in soil infested with F. oxysporum f. perniciosum (Hepting) Toole and Eitner M. incognita or M. javanica, there was severe wilting of mimosa seedlings.

Sumner and Johnson (1973) studied the effect of root-

knot nematodes on Fusarium wilt of watermelon caused by F. oxysporum f. sp. niveum (E.F.Sm.) Snyder and Hansen and found that wilting was more in presence of M. incognita. They also reported that wilt symptoms were more severe in resistant than in susceptible cultivars. Giamalva et al. (1962), on the other hand, failed to observe significant effect on Fusarium wilt of sweet potato caused by F. oxysporum f. bataius (Wr.) Snyder and Hansen.

Association of M. incognita and F. oxysporum var. lycopersici, causal organism of wilt of okra, caused high seedling mortality and root-knot index on okra than either of the pathogens alone (Khan and Saxena, 1969).

There are innumerable reports wherein Verticillium, another wilt causing fungus, develops synergistic relationship with a variety of nematodes, as for example V. albo-atrum Reinke and Berthold with Pratylenchus penetrans on eggplant (McKeen and Mountain, 1960), tomato (Conroy et al. 1972) potato (Morsink and Rich, 1968; Burpee and Bloom, 1974), peppermint (Bergeson, 1963), pepper (Olthof and Meyers, 1969) and Impatiens (Muller, 1972); with Tylenchorhynchus capitatus Allen, 1955 and M. incognita (Overman and Jones, 1970), Trichodorus christiei Allen, 1957 (Conroy and Green, 1974) and Heterodera tabacum (Miller, 1975) on tomato; V. dahliae Aleb. with P. penetrans on tomato (Mountain and McKeen, 1962) and with P. minvus Sher and Allen, 1953 on

peppermint (Faulkner and Skotland, 1965).

II. NEMATOLE-FUNGUS ROOT-ROT COMPLEXES

Casser et al. (1955) observed that the presence of root-knot nematode reduced the resistance in tobacco varieties to black shank fungus Phytophthora parasitica f. nicotianae. Powell and Nusbaum (1958, 1960) while studying the interaction between P. parasitica f. nicotianae and M. incognita acrita reported that the fungus colonized the galled tissue resulting in drastic tissue disorder. Similar results have been obtained by Miller (1968) with M. javanica and P. parasitica f. nicotianae on tobacco variety NC 95. and by Inagaki and Powell (1969) with root lesion nematode, Pratylenchus brachyurus and P. parasitica f. nicotianae on black shank susceptible tobacco varieties. Simultaneous inoculation of nematode and fungus or when nematode preceded the fungus by one week resulted in more severity than when nematodes were inoculated 3-4 weeks in advance. Powell and Batten (1967) and Melendez and Powell (1969) found that tobacco seedlings and even mature plants were least affected by M. solani Kuhn, Pythium ultimum Trow and Trichoderma harzianum Rifai, however, they caused extensive damage even to mature plants which were already infected with M. incognita. However, simultaneous inoculation with the above fungi and root-knot resulted in

little damage. Again Melendez and Powell (1970) reported that M. incognita predisposed flue-cured tobacco roots to Pythium ultimum and damage to roots was more when the nematode preceded the fungus. Powell et al. (1971) observed an interesting disease complex between soil inhabiting fungi (Pythium, Curvularia, Botrytis, Aspergillus, Penicillium and Trichoderma) and root-knot M. incognita on flue-cured tobacco cultivar C 316 and found that severe necrosis on tobacco roots occurred when the nematodes preceded the fungi by several weeks but none of the fungi induced disease in the absence of M. incognita.

Lunn and Hughes (1964) and Dunn (1968, 1970) found more reduction in growth of tomato when Heterodera rostochiensis entered the roots before G. solani and Colletotrichum atramentarium (Berk. and Br.) Taub. than when fungus preceded the nematode or when the two pathogens entered simultaneously. A disease complex between root-knot nematode, M. incognita and root-rot fungus, G. solani on okra and tomato was also reported by Golden and Van Gundy (1975).

Goswami et al. (1970) observed that 25% plants wilted when brinjal seedlings were inoculated with M. incognita and Sclerotium rolfsii Sacc. as against 6% when inoculated with fungus alone. The root-rot of eggplant caused by either

G. solani, Pythium spp. or Colletotrichum atramentarium was aggravated in the presence of M. incognita and the damage to the eggplants was more when nematode preceded the fungus (Azam, 1975).

Wyllie and Taylor (1960) found that in soybean, root-rot caused by Phytophthora sojae Kaufmann and Gerdemann was more severe in presence of M. hapla rather than in its absence and the damage was related to soil temperature. Hedrick and Southards (1976) observed heavy damage of soybean by Cylindrocladium crotolariae in the presence of M. incognita. More damage occurred when nematode preceded the fungus.

Irvine (1965) reported that at 20°C to 30°C maximum number of alfalfa plants were killed when infected with M. hapla and G. solani while at 15°C no significant reduction in yield of alfalfa occurred. G. penetrans and Trichoderma viridi Pers. ex. Fr. in association caused more reduction in root and shoot growth in alfalfa than either of the organisms alone (Edmunds and Mai, 1966).

Haglund and King (1961) noticed that Tylenchorhynchus martini Fielding, 1956 increased the severity of root-rot of pea caused by Aphanomyces euteiches Drechsler and this increase was directly related to the population of the nematode.

Minton and Jackson (1967) reported the increased invasion of Aspergillus flavus Link. on pea nuts in presence of H. arenaria or H. hapla. Jackson and Minton (1968) observed no effect of A. flavus or A. niger Van Tieghem in the presence of lesion nematode Pratylenchus spp. The incidence of pod-rot of pea nut was more severe when Pythium myriotylum Drechs. and Fusarium solani or H. arenaria were present together than P. myriotylum alone (Garcia and Mitchell, 1975).

Grainger and Clark (1963) observed a considerable decrease in potato yield when potatoes were moderately infested with H. solani and Heterodera rostochiensis.

Price and Schneider (1965); Polychronopoulos et al. (1969) and Polychronopoulos (1970) reported that Heterodera schachtii Schmidt, 1871 and H. solani together considerably promoted root and seedling damage in sugarbeet. Jorgenson (1970) observed the antagonistic interaction between H. schachtii and F. oxysporum on sugarbeet as the damage to sugarbeets was less when the fungus and nematode were present than when nematode only was present.

Apt and Koike (1962) reported that presence of H. incognita acrita and root-rot fungus Pythium graminicola Subram. reduced top growth of sugarcane but not the root growth while Santo and Holtzmann (1970) reported that

simultaneous inoculation with Pratylenchus zeae Graham, 1951 and Pythium graminicola reduced both top and root growth more than either of the pathogen alone. Damage aggravated when nematode preceded the fungus.

Littrell and Johnson (1969) reported that "Ice berg" chrysanthemum plants, when inoculated with Pythium aphanidermatum (Edson) Fitzp. and Helonolaimus longicaudatus or H. incognita, developed symptoms of root-rot earlier and more extensively than those inoculated with the fungus alone. H. longicaudatus and Pythium combination was more severe than H. incognita and Pythium. Most severe reduction in plant growth occurred when both the nematodes were combined with Pythium during inoculation (Johnson and Littrell, 1970).

O'Bannon et al. (1967) reported an increase in root decay by Fusarium spp. in lemon if Tylenchulus semipenetrans is also associated.

Palmer et al. (1967) reported that at certain temperature Pratylenchus scribneri Steiner, 1943 and Fusarium moniliforme Sheldon caused greater reduction of corn as compared to either of the pathogens alone. Kisiel et al. (1969) noticed that Tylenchus agricola (de Man, 1884) Filipjev, 1934 also contributed towards the increase in root-rot of corn caused by F. roseum (Link.) emended Nyder and Hansen. A synergistic interaction between H. incognita

and F. moniliforme on maize has also been reported by Palmer and Mac Donald (1974).

Mountain and Benedict (1956) reported that Pratylenchus minvus and Rhizoctonia solani in combination reduced winter wheat growth two times to that by either of the pathogens alone.

III. NEMATODE-FUNGUS SEEDLING DISEASE COMPLEXES

It is difficult to separate root-rots and seedling diseases because the later are nothing but are reflections of root disorders. There are large number of examples where nematode fungus combinations resulted in greater damage to seedlings.

Reynolds and Hanson (1957) reported that post-emergence damping-off in cotton, caused by Rhizoctonia solani, became severe in presence of M. incognita acrita while Norton (1960) observed greater pre-emergence damping-off in cotton caused, by R. solani, F. oxysporum f. vasinfectum and Pythium debaryanum Hesse, in the presence of M. incognita acrita. A synergistic interaction between R. solani, P. debaryanum and root-knot nematode in seedling disease complexes of cotton was reported by White (1962) and Brodie (1963). R. solani caused more destruction in cotton seedlings when they were grown in soil infested with Motylen-

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chulus reniformis or Hoplolaimus tylenchiformis Daday, 1905 or M. incognita or M. hapla or M. arenaria (Brodie and Cooper, 1964).

Taylor and Wyllie (1959) reported that the association of M. javanica or M. hapla with G. solani adversely affected the emergence of soybean seedlings. The simultaneous inoculation with M. incognita and Macrophomina phaseoli (Maubley) Ashby or when nematode preceded the fungus resulted in greater seedling mortality of soybean than when fungus preceded the nematode or fungus alone was used (Agarwal and Goswami, 1973). The association of Heterodera glycines and Phytophthora me. asperma Drechs. var. soiae Hild caused greater damage to soybean seedlings than either of the pathogens alone (Adeniji et al., 1975).

Whitney (1971, 1974) reported synergism between H. schachtii and Pythium ultimum in pre and post-emergence damping-off of sugarbeet seedlings.

Synergism between root-knot nematode and Sclerotium rolfsii in emergence of tomato seedlings was reported by Chukla and Swarup (1970).

Khan et al. (1971) reported much greater reduction in the emergence of cauliflower seedlings in the presence of Tylenchorhynchus brassicae Siddiqi, 1961 and G. solani than in the presence of G. solani alone.

Wehant and Weaber (1972) found the growth reduction in peach seedlings when Fusarium oxysporum was present along with either Hoplolaimus galeatus or Tylenchorhynchus claytoni Steiner, 1937 or Creconemoides xenoplex Askani, 1952.

Kaman et al. (1974) observed that seedling blight of rice caused by Sclerotium rolfsii was much greater in presence of Hoplolaimus indicus Sher, 1963.

Legumes are cultivated all over the world and stand next to cereals in their economic importance (Allen and Allen, 1958). In India, legumes are grown almost in all the states either as alone or mixed with other crops. Besides their food value many legumes are cultivated for fodder purposes. Legumes are attacked by large number of wilt and root-rot organisms causing heavy toll of the crop every year. During a survey of legume fields at Indian Grass-land and Research Institute, JHANSI, in cowpea, Vigna ^uundiculata fields severe seedling mortality and stunting of plants was noticed in some beds. The location of these patches did not seem to be associated with any observable differences in soil condition. A close examination of infected roots revealed the existence of lesions and galls, the former caused by Rhizoctonia solani and the later by root-knot nematode Heloidoxyne incognita.

In view of the observations it has been desired to study the following:-

- (i) Screening of different cowpea cultivars against Meloidogyne incognita (Kofoid and White, 1919) Chitwood, 1949 and Rhizoctonia solani Kuhn with a view to study their response under monopathogenic situations.
- (ii) Response of resistant and susceptible cultivars obtained in (i) to the combined effect of M. incognita and M. solani by inoculating at different time intervals, studying if there is any bio-predisposition.
- (iii) To study the changes in morphology, biology and population of the pathogens.
- (iv) To study the differences in host response resulting from inoculation under monopathogenic conditions and comparing it when the plants have been inoculated with root-knot and R. solani.

M A T E R I A L S A N D M E T H O D S

RAISING CULTURES AND THEIR MAINTAINANCE

RHIZOBIA

Fresh culture of cowpea rhizobia will be obtained from the Division of Microbiology, Indian Agricultural Research Institute, New-Delhi. The rhizobial culture so obtained will be diluted in sterilized water in order to obtain suspension having 0.500 optical density. Seedlings will be inoculated with 10 ml. of this suspension per plant.

NEMATODE

The inoculum of root-knot nematode, Meloidogyne incognita will be raised on tomato seedlings from single eggmass collected from infected roots of tomato. The eggmass will be surface sterilized by treating with 1.500 aqueous solution of chlorex (deWouden, 1958) and will be washed thrice in sterilized distilled water. The eggs will be allowed to hatch into larvae under aseptic conditions in a sieve, layered with tissue paper in petridish containing sufficient amount of sterilized water. The tomato seedlings raised in autoclaved soil will be inoculated with these larvae. In order to have regular supply of the root-knot larvae, tomato seedlings will be inoculated at different intervals from time to time.

FUNGUS

Rhizoctonia solani will be isolated from the infected roots of cowpea and pure culture will be maintained on potato-dextrose agar.

Inoculum of the fungus will be raised in Richard's solution (Potassium nitrate, 10.0 g; Potassium dihydrogen phosphate, 5.0 g; Magnesium sulphate, 2.5 g; Ferric chloride, 0.02 g; Sucrose, 50.0 g and distilled water, 1,000 ml) contained in 250 ml Erlenmeyer flasks. The mycelial mat will be washed in distilled water and blended for 30 seconds. Fungal suspension will be prepared by mixing the fungal mycelium in distilled water at the rate of 10 g of mycelium in 100 ml of sterilized distilled water.

RAISING SEEDLINGS OF COWPEA

Surface sterilized seeds (by treating with 95% alcohol and washing in sterilized water) of different cultivars of cowpea to be used will be allowed to germinate on moistened sterilized filter paper, placed in sterilized petriplates. Seedlings of uniform size will be singly transplanted to autoclaved soil, contained in 15 cm clay pots.

INOCULATION TECHNIQUE

There shall be following treatments:-

1. Seedlings without Rhizobium.
2. Seedlings inoculated with Rhizobium.
3. Seedlings inoculated with M. incognita.
4. Seedlings inoculated with Rhizobium + M. incognita.
5. Seedlings inoculated with G. solani.
6. Seedlings inoculated with Rhizobium + G. solani.
7. Seedlings inoculated with M. incognita + G. solani.
8. Seedlings inoculated with Rhizobium + M. incognita + G. solani.

For inoculating with nematodes a suspension of root-knot nematode larvae will be made in water so as to contain 2,000 larvae per 10 ml of suspension by using counting dish (Southey, 1970). This suspension will be poured around the plant near the roots by making four holes at equal distance.

For inoculating with the fungus, aqueous suspension of mycelium of G. solani will be made so as to contain 1 g mycelium per 10 ml of water. 20 ml of this suspension (containing 2 g of fungus) will be poured around the root zone by removing a portion of the soil.

In inoculation with root-knot nematode and fungus,

both inocula will be poured simultaneously around the root zone. The seedlings will also be inoculated with nematode 15 and 30 days prior to fungal inoculation and vice-versa.

SCREENING OF CULTIVARS

Preliminary screening of about 40-50 different fodder cultivars of cowpea obtained from I.G.F.R.I., Jhansi, will be made against M. incognita and A. solani.

For screening against M. incognita, surface sterilized seeds of different cultivars will be sown in small earthen pots containing 150 g washed autoclaved sand. On emergence seedlings will be inoculated with 500 larvae of root-knot nematode. The plants will be given nutrient solution (Long-Ashton) once a day. After 7, 14 and 21 days of inoculation seedlings will be uprooted and thoroughly washed in running water and the roots will be stained with acid fuchsin and cleared in lectophenol for 24 hours. The number of larvae in different stages inside the roots will be counted by teasing the roots under the stereoscopic microscope. On the basis of number of larvae present in different stages in the roots the cultivars will be rated as follows:-

	<u>% of larvae penetrated</u>	<u>% of larvae developed in III & IV stage</u>
1. Highly resistant	0-10	0-10
2. Resistant	10-30	10-30
3. Susceptible	30-50	30-50
4. Highly susceptible	More than 50	More than 50

For testing against R. solani surface sterilized seeds of different cultivars of cowpea will be placed on a small platform of filter paper strip fitted nicely in a sterilized test tube containing 3 ml of distilled water. The seeds on germination will be inoculated with equal amount of concentrated aqueous solution of mycelium (Simmonds and Sallans, 1946). Resistance and susceptibility will be determined by calculating rot/root ratio as under -

$$R/R \text{ Ratio} = \frac{\text{Length of infected root (L}_I\text{)}}{\text{Total length of the root (L}_T\text{)}}$$

On the basis of rot/root ratio the cultivars will be categorised as -

	<u>R/R Ratio in percent</u>
1. Highly resistant	Less than 5
2. Resistant	5 - 20
3. Susceptible	20 - 70
4. Highly susceptible	More than 70

Out of the different cultivars tested above one cultivar each of the above different categories (1, 2, 3 and 4) against nematode and fungus will be selected for further study. The seedlings will be raised in autoclaved soil and will be inoculated with root-knot nematode or fungus (under monopathogenic condition). After 45 days plants will be uprooted and degree of nodulation, root-knot index in addition to fresh and dry weight of plants will be determined.

HISTOPATHOLOGICAL STUDIES

To study the host response^s resulting from individual and combined inoculations with root-knot nematode and Rhizoctonia, cowpea roots after 5, 10, 20, 30 and 45 days of inoculation and corresponding healthy roots will be killed and fixed in F.A.A. for 48 hrs. and will be stored in 70% alcohol. The root pieces will be processed through upgrade series of alcohol and will be brought in absolute alcohol. After passing through different grades of alcohol and xylol, the root pieces will be embedded in molten paraffin wax of 58°C. The paraffin embedded material will be sectioned at 20 μ . The sections will be stained with safranin and fastgreen (Johanson, 1940). The slides will be examined under microscope and changes in the anatomy of host will be noted.

RECORDING OF DATA

PLANT GROWTH DETERMINATION

Except otherwise mentioned, 60 days after inoculation the plants will be uprooted and thoroughly washed in running water. The excess of water will be removed by using blotting paper and utmost care will be taken not to disturb the root system during entire operation. The growth of plants will be determined by measuring length, fresh and dry weight of root and top.

ROOT-NODULE ESTIMATION

Masefield (1958) pointed out that two figures mainly number and weight of nodules, the total weight of nodules is more important, for the fresh weight can be shown to be highly correlated with the volume of bacterial tissue and this volume determines, together with longevity, the possible amount of nitrogen fixation. Therefore, the nodulation will be estimated by weighing nodules/gm. of root and will be expressed in terms of nod/root ratio.

ROOT-KNOT ESTIMATION

Intensity of root-knot will be rated on the basis of following scale:-

0 = Immune

1 = Few galls without egg masses

- 2 = Few galls with egg masses
- 3 = Moderately heavy galling with egg masses
- 4 = Severe galling with numerous egg masses.

NEMATODE POPULATION ESTIMATION

Population estimation will be done by counting the number of eggs and larvae in root (by macerating 1 g of root from each replicate in 4% NaCl^O) and larvae in the soil.

The morphology of females and males will be determined in detail. For females perinial patterns will be cut from stained material and its variation will be determined. The stylet length dorsal gland opening and the location of excretory pore will also be determined. For larvae tail length, tail tip, total length, inflated or dialated rectum characters will be studied and measured. In case of males stylet length, head annules and lateral field will be studied.

Study of variation in the morphology of genus based upon the above characters as a result of the presence of A. solani will be of great importance.

The data obtained will be analysed statistically.

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